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# Latex Immunoassay of Urinary Albumin

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**Summary:** A new non isotopic immunoassay based on latex particle agglutination has been developed for the determination of albumin in urine. The assay consists in incubating the urine sample for 30 min at 37 °C with latex particles coated with a specific antibody, followed by quantification of the resulting agglutination by particle counting or turbidimetry. The stability of antibody-coated particles during incubation is achieved by diluting them just before the assay in a buffer containing bovine serum albumin. The pH of this buffer, the antibody loading and the concentration of latex particles in the incubation mixture were optimized to obtain the maximal specific and minimal nonspecific agglutination.

The albumin standard curve ranges from 25 to 800 µg/l. Reproducibility tests performed within and between run gave CV's ranging from 8.2 to 11.6%. The recovery of albumin added to 10 urines averaged 95.5%. A correlation coefficient of 0.96 was obtained between latex immunoassay and nephelometry for the determination of albumin in 51 urines.

*Latex-Immunassay für Albumin im Harn*

**Zusammenfassung:** Ein neuer Immunassay ohne Verwendung radioaktiver Isotope auf der Grundlage der Latexpartikel-Agglutination für die Bestimmung von Albumin im Harn wurde entwickelt. Das Verfahren besteht in der Inkubation der Harnprobe für 30 min bei 37 °C mit Antikörper-beschichteten Latexpartikeln, gefolgt von der Quantifizierung der sich ergebenden Agglutination durch Partikelzählung oder Turbidimetrie. Die Stabilität der Antikörper-beschichteten Partikel während der Inkubation wird dadurch erreicht, daß sie kurz vor Durchführung der Bestimmung in einem Puffer, der Rinderserum-Albumin enthält, verdünnt werden. Der pH-Wert dieses Puffers, die Antikörper-Beladung und die Konzentration der Latexpartikel im Ansatz wurden optimiert, um die maximale spezifische und minimale nicht-spezifische Agglutination zu erreichen.

Der Bereich der Standardkurve für Albumin reicht von 25 bis 800 µg/l. Die Untersuchung der Reproduzierbarkeit in der Serie und von Tag zu Tag ergab Variationskoeffizienten von 8,2–11,6%. Zu 10 Harnen zugefügtes Albumin wurde zu 95% wiedergefunden. Zwischen Latex-Immunassay und Nephelometrie für die Bestimmung von Albumin in 51 Harnen wurde ein Korrelationskoeffizient von 0,96 erhalten.

## Introduction

Increased urinary excretion of high molecular weight proteins such as albumin constitutes an early sign of glomerular dysfunction induced by many nephrotoxic agents. The immunological methods currently available for measuring albumin in urine usually have satisfactory sensitivity. However, techniques involving immunoprecipitation in gel, such as radial immunodiffusion (1) or electroimmunoassay (2), are not suitable for routine analysis, being laborious and time consuming.

Several radioimmunoassay methods have been described for the determination of albumin in urine but these also have disadvantages, such as long incuba-

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tion time, short shelf life of the reagents and cumbersome labelling procedures (3–6). The nephelometric assay of albumin, as applied in the Automated Immunoprecipitin System (Technicon, Tarrytown, N.Y. USA) is certainly a method of choice because it is sensitive and automated (7). The latex immunoassay (LIA) method described in this paper is based on the agglutination by albumin of anti-albumin-coated latex particles. The agglutination is quantified either by particle counting as first proposed by *Cambiaso* et al. (8), or by turbidimetry. LIA is particularly suitable for routine screening of abnormal albuminuria: it is simple, fast, sensitive, accurate and consumes very small quantities of antibody. Furthermore, it can be performed with the same equipment and reagents as those used in a recently developed LIA of $\beta_2$-microglobulin (9). The LIA of albumin in urine, combined with that of $\beta_2$-microglobulin represents a simple and rapid method for the early detection of renal damage and for the differential diagnosis of tubular, glomerular or mixed type proteinuria. It is therefore suitable for the routine health surveillance of workers exposed to nephrotoxic chemicals.

## Materials and Methods

### Reagents

Human serum albumin (Fluka AG, Buchs, Switzerland) was used as a standard. Its concentration was determined by measuring the absorbance at 280 nm ($A^{1\%}_{280\,nm} = 5.8$). Rabbit anti-albumin immunoglobulin was supplied by Dako Immunoglobulin (code No. 10–061, Copenhagen, Denmark). The immunoglobulin concentration estimated from the absorbance at 280 nm ($A^{1\%}_{280\,nm} = 13.8$) was 15.6 g/l.

Polystyrene latex particles, 0.79 µm diameter were kindly supplied as a 10% suspension by Dr. *J. C. Daniel* of Rhône-Poulenc (Aubervilliers, France) (Estapor K109, lot 510 and 350). The glycine-buffered saline used throughout the assay was prepared by the 10-fold dilution of a stock buffer (1 mol/l glycine, 1.7 mol/l NaCl and 76 mmol/l $NaN_3$, pH adjusted to 9 with 10 mol/l NaOH).

### Urine samples

Urine specimens were collected from 50 apparently healthy male subjects aged 20 to 60 years, and from 51 male workers employed in a cadmium-producing plant. The urines were stored at − 18 °C with 15 mmol/l $NaN_3$.

### Latex immunoassay

*Coating of latex particles with antibody*

The procedure used for adsorbing the anti-albumin antibody on latex particles is similar to that described in the LIA of $\beta_2$-microglobulin (9): fifty microliters of the 10% latex suspension are mixed in 0.4 ml glycine buffered saline with an aliquot of the antibody solution (usually 10 microliters). After about 1 h incubation at room temperature, the particles are precipitated by centrifugation (25000 *g*, 10 min, 25 °C), washed twice with 1 ml of a 0.1 mol/l NaCl and 15 mmol/l $NaN_3$ solution and finally resuspended in 1 ml of this solution. When kept at 4 °C, this preparation can be used for at least 6 months.

*Preparation of stabilization solution*

Latex particles coated only with antibody are considerably unstable and agglutinate spontaneously. This nonspecific agglutination can be overcome by introducing negative charges on the latex surface. In the previously published LIA for $\beta_2$-microglobulin, this was realized by diluting the antibody-coated particles, just before the assay, in a glycine buffered saline buffer (10 g/l of bovine serum albumin (bovine serum albumin, pH 7, Fluka), adjusted to pH 10 and filtered through a 0.45-µm Millipore membrane). However, when stored at alkaline pH, bovine serum albumin progressively loses its stabilizing properties. Therefore, another procedure has been introduced for the preparation of this solution. A distilled water solution containing 11 g/l of bovine serum albumin is prepared, divided into aliquots and stored frozen or at 4 °C with 15 mmol/l $NaN_3$. A volume of the glycine buffered saline stock buffer (1 mol/l glycine, 1.7 mol/l NaCl and 76 mmol/l $NaN_3$, pH 9) is brought to pH 10, filtered through a 0.45-µm Millipore membrane and stored at 4 °C. For the stabilization of antibody-coated particles for the assay (see below), the stabilization solution is reconstituted by mixing the albumin solution and the glycine buffered saline stock buffer at pH 10 in the proportions 9 + 1 (by vol.).

*Assay procedure*

The assay is very similar to that described previously for $\beta_2$-microglobulin (9). Dilutions of albumin standards and of urines are made in glycine buffered saline containing 1 g/l of bovine serum albumin and filtered through a 0.45-µm Millipore membrane (glycine buffered saline/bovine serum albumin). Aliquots of 20 microliters of albumin standard or diluted urine samples are pipetted in duplicate into glass test tubes. Tubes containing aliquots of glycine buffered saline/bovine serum albumin are regularly spaced in each series of analyses (zero standard). Antibody-coated latex particles are stabilized as follows: disperse the required volume of antibody-coated particles in the 11 g/l bovine serum albumin solution. Sonicate (Branson B12, sonifier) or vigorously vortex-mix the suspension for 5 minutes in order to destroy aggregates formed during storage. Then while sonicating add the glycine buffered saline stock buffer pH 10 and further sonicate a few seconds to ensure homogenization. The volumes of the albumin solution and of the glycine buffered saline stock buffer pH 10 (mixed in the proportions 9 + 1 (by vol.) to reconstitute the stabilization solution) are usually adjusted to give a 5-fold dilution of the antibody-coated latex particles. Fifty microliters of stabilized latex are then added to each tube. After a rapid vortex-mixing, the mixture is incubated for 30 min at 37 °C in a shaking water bath (80 $min^{-1}$, 5.5 cm amplitude). The incubation is stopped by adding 5 ml of glycine buffered saline containing 1 ml/l of Tween 20 previously filtered through a 0.45-µm Millipore membrane. Latex particle aggregates formed during incubation are very stable. The tubes may be stored 12 h at 25 °C or 24 to 48 h at 4 °C without significant modification in the agglutination.

*Reading*

The agglutination was quantified either automatically by particle counting or manually by turbidimetry. The particle counting measurement was performed automatically with a Technicon Autocounter (Technicon Corp. Tarrytown, N.Y.) as described previously (9). In the turbidimetric method, the decrease of the absorbance at 360 nm (2 cm cuvette) is measured with a Zeiss PMQ II spectrophotometer (Oberkochen, West Germany). Before reading, the latex suspension must be homogenized by gently inverting the tubes several times. With the turbidimetric method, this operation must be carried out for each tube individually be-

fore transferring its content in the cuvette. As shown previously for $\beta_2$-microglobulin LIA, the particle counting and the turbidimetric methods are comparable with respect to their precision and accuracy (10).

*Calculation of the results*

When the reading is performed by particle counting, the peak height of each analysis is expressed as a percentage of the mean peak height of the zero standard. A slight shift (1–2%) may be observed in the peak height of the zero standard. In that case, the zero standard peak corresponding to each analysis is estimated by linear extrapolation. Providing this shift does not exceed about 10%, the standard curve is not significantly affected.

In the turbidimetric reading, the agglutination is expressed as the difference between the absorbance of the zero standard and that of the analysis. Normally, there is no detectable drift in the absorbances of the zero standard, but if this does occur, then the results are calculated as for particle counting. It may be useful to also calculate the percentage of nonspecific agglutination occurring in the zero standards during the incubation, this parameter being an indicator of the stability of the antibody-coated particles. For this purpose, two 50 µl aliquots of stabilized particles are diluted with 5 ml of glycine buffered saline containing 1 ml/l of Tween-20 just before incubation. The difference between the mean absorbance of these tubes and that of the zero standard represents the nonspecific agglutination.

Other methods

The nephelometric assay of albumin in urine was performed by the Automated Immunoprecipitin technique of *Ritchie* et al. (11).

## Results

*Comparison of turbidimetric and particle counting reading*

In the LIA of albumin, the agglutination curves obtained by both methods are very similar (fig. 1). No difference was observed in the values of urinary albumin determined by both techniques. A correlation coefficient of 0.98 with a regression coefficient close to 1 was obtained between turbidimetry and particle counting when 21 normal urines with albumin concentrations ranging from 0.71 to 11.4 mg/l were analysed.

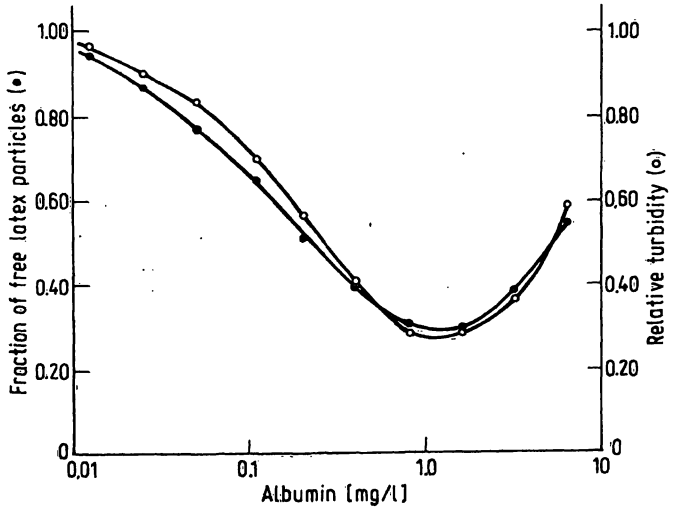


Fig. 1. Standard curves of albumin obtained by particle counting (●) or by turbidimetry (O).

*Effect of antibody loading and of latex particle concentration*

Increasing the amount of antibody adsorbed on the latex particles has 3 effects (fig. 2):

1. an increase of the specific agglutination;
2. a reduction of the postzone effect;
3. an increase of the instability of the particles which results in a greater nonspecific agglutination.

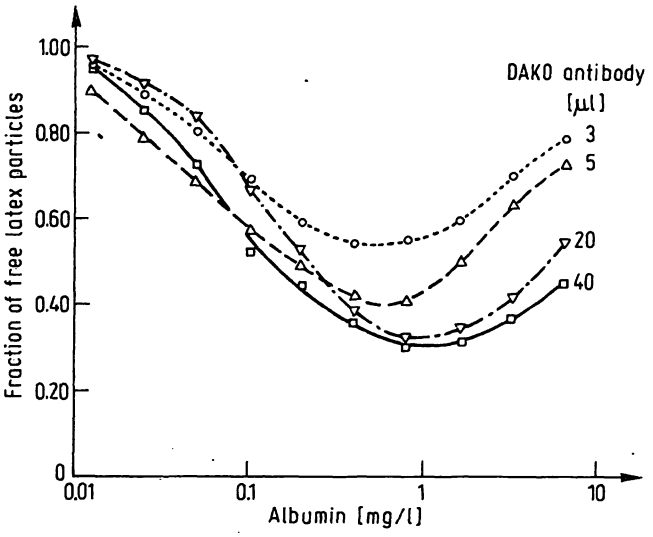


Fig. 2. Effect of antibody loading of latex particles on their agglutinability by albumin. The volumes of DAKO antibody solution adsorbed on 50 microliters of a 10% suspension of latex particles are indicated. The antibody-coated particles were diluted 5 times with the stabilization solution.

Similar effects were observed when the concentration of latex particles in the incubation mixture was increased by modifying the dilution rate with the stabilization solution (fig. 3). A maximum of specific agglutination was reached with an antibody loading of 10 microliters per 50 µl of the 10% latex suspension and a 5-fold dilution with the stabilization solution (fig. 3). Attempts to shift the postzone effect outside the biological range of albumin concentration in urine were unsuccesful; with an antibody loading of 40 µl per 50 µl latex, the instability of the particles was already so high (nonspecific agglutination > 50%) that no reproducible specific agglutination could be obtained. Urines are therefore systematically analysed at two different dilutions to avoid erroneous results.

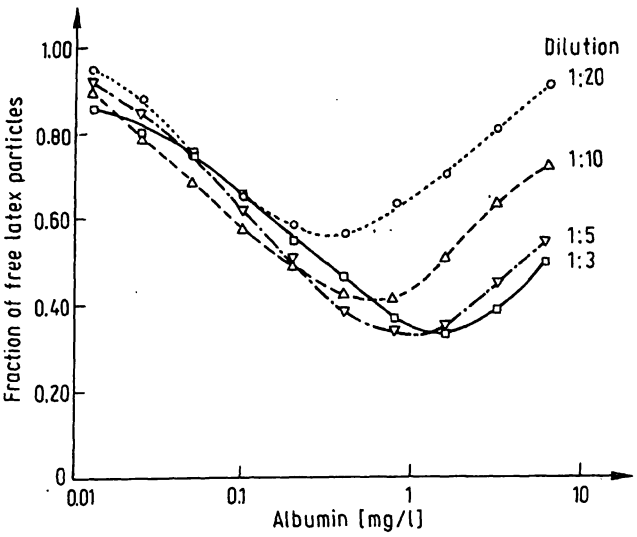


Fig. 3. Effect of concentration of antibody-coated latex particles on their agglutinability by albumin. The particles were diluted 3 to 20 times with the stabilization solution. Their antibody loading was 10 microliters DAKO antibody per 50 microliters of a 10% latex suspension.

### *Effect of pH of the stabilization solution*

Like the antibody loading and the concentration of latex particles, the pH of the stabilization solution and hence that of the incubation mixture (about 0.2 pH unit lower than the former) also has an effect on both specific and nonspecific agglutination (fig. 4). The specific agglutination increases with the pH of the stabilization solution to reach a maximum at pH 10. Above this pH value, a progressive decrease in the specific agglutinability is observed. On the other hand, increasing the pH continuously improves the stability of the particles, so that pH 10 appears as an optimum.

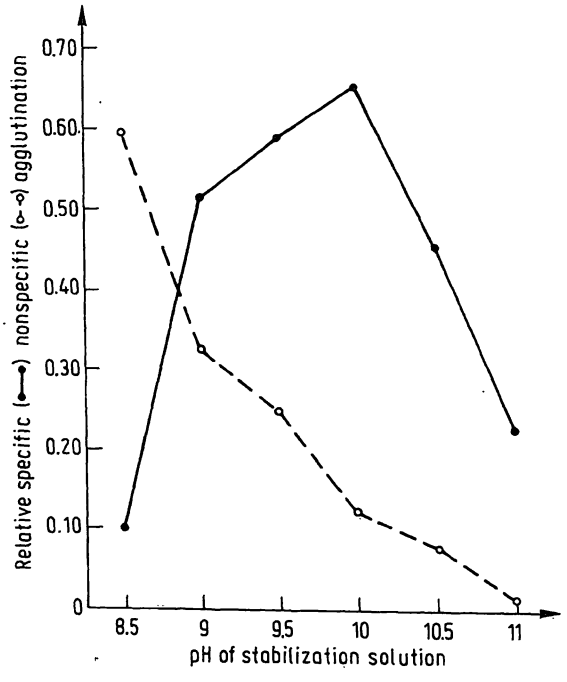


Fig. 4. Effect of pH of the stabilization solution on the specific and nonspecific agglutinability of anti-albumin antibody-coated particles (10 µl DAKO antibody per 50 microliters of the 10% latex suspension, 5-fold dilution with the stabilization solution).

### *Specificity*

The concentration of albumin was measured by LIA in 5 urines with different albumin concentrations. From figure 5, it can be seen that albumin levels vary linearly with the urine dilution and that the regression lines have the same slope.

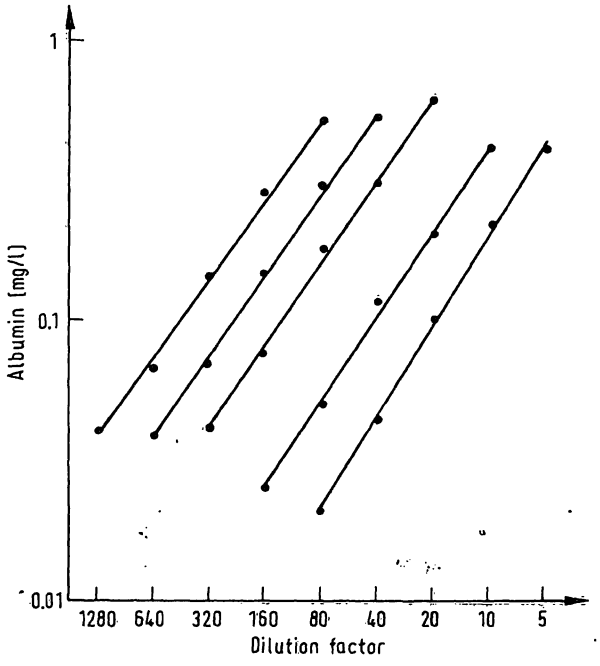


Fig. 5. Concentrations of albumin obtained by serial dilutions of 5 different urines.

### *Sensitivity*

An albumin concentration of 10 µg/l, which represents approximately 0.2 ng or 3 fmol of albumin, reduces the number of free latex particles or the turbidity by about 10% (figs. 2 and 3). This sensitivity is comparable to that reached with radioisotopic methods.

### *Accuracy*

Albumin was added to 10 different urines so that their albumin concentrations were increased by 10 mg/l. Analytical recovery averaged 95.5% (SE = 2.9) with a range of 80.5 to 107.4%.

*Reproducibility*

Within- or between-run precisions were tested on 10 duplicate determinations of albumin carried out during one month in a pool of normal urines diluted 25, 50 and 100 times. The CV ranged from 8.2 to 11.6% (tab. 1).

Tab. 1. Within and between assay reproducibility of 10 determinations by latex immunoassay of albumin concentration (mg/l) in a pool of normal urines.

| Dilution | Within assay | | | Between assay | | |
|---|---|---|---|---|---|---|
| | Mean | SD | CV (%) | Mean | SD | CV (%) |
| 1/25 | 4.83 | 0.48 | 10 | 5.68 | 0.48 | 8.8 |
| 1/50 | 5.44 | 0.43 | 9 | 5.71 | 0.43 | 8.2 |
| 1/100 | 5.52 | 0.64 | 11.6 | 5.54 | 0.64 | 11 |

There is, however, a relationship between the reproducibility of the assay and the stability of the antibody-coated particles. The above-mentioned CV's were obtained with a nonspecific agglutination below 10%, but when particles are excessively unstable (for instance nonspecific agglutination higher than 50%), the reproducibility of the assay decreases drastically.

*Comparison with nephelometry*

The concentration of albumin in 51 urines with normal or slightly increased albuminuria were measured by LIA and nephelometry (A.I.P. system, Technicon). An excellent correlation was observed between both methods, although the nephelometric assay tends to slightly overestimate the values of albuminuria in the low concentration range. This is probably due to the lack of accuracy of nephelometry in the estimation of low albumin values (limit of detection around 1 mg/l). LIA is as accurate at low as at high albumin values (fig. 5).

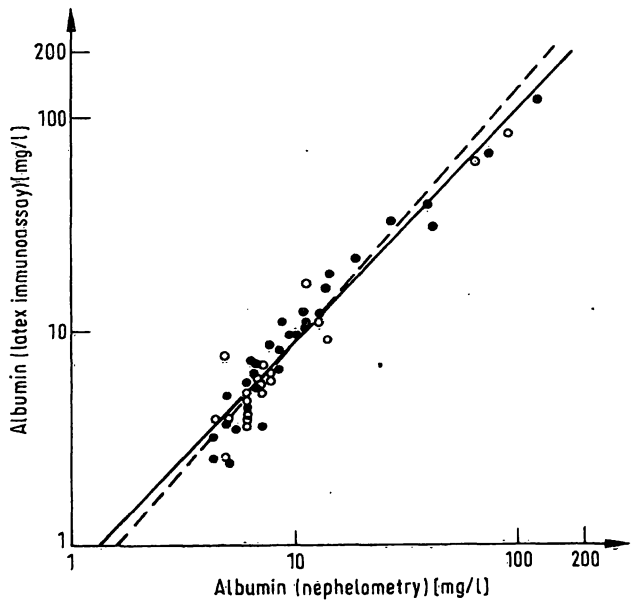


Fig. 6. Correlation between latex immunoassay and nephelometry for the determination of albumin in 51 urines from workers non exposed (O) or exposed (●) to cadmium. The equation of the regression line is y: −0.15 + 1.10 x.

*Reference values*

In spot urine samples collected from 50 apparently healthy subjects, the mean urinary concentration of albumin was 6.82 mg/l (SD = 5.73; range = 0.49–34.2) or 4.43 mg/g creatinine (SD = 2.98; range = 1.29–18.4).

## Discussion

In the development of LIA for albumin and also for $\beta_2$-microglobulin (9), the main difficulty to be overcome was the control of the stability (or nonspecific agglutination) of the antibody-coated particles. From our experience with LIA, it seems that to obtain a reasonable sensitivity and precision, the nonspecific agglutination occurring during incubation must not exceed about 10%. Therefore, the optimal conditions of the assay must be defined to obtain the maximum specific agglutination but with a nonspecific agglutination less than 10%. Factors such as the antibody loading, the concentration of particles in the incubation mixture, the pH of the stabilization solution or the duration of incubation determine the specific agglutinability of the antibody-coated particles and their stability during incubation. In the LIA of $\beta_2$-microglobulin described previously (9), we determined the conditions for a very sensitive agglutination curve with less than 2% of instability. However, in the LIA of albumin, the nonspecific agglutination is about 10% (fig. 4). This difference is probably due to a higher antibody-loading of the latex particles. It is clear that the difficulties encountered in the attempt to stabilize the antibody-coated particles, originated from the instability of the stabilization solution itself. When stored at alkaline pH, bovine serum albumin progressively loses its stabilizing properties. This difficulty was overcome by increasing the pH of the albumin solution immediately after coating of the latex particles with bovine serum albumin.

The stability of antibody-coated latex particles is also a prerequisite for automating the measurement. Recently, the LIA of $\beta_2$-microglobulin, retinol-binding protein and albumin has been automated in our

laboratory with an excellent precision and sensitivity, simply by using an incubation of about 25 min in a mixing coil, and by quantifying the agglutination with a Technicon Autocounter (PACIA, 8) (*Bernard & Lauwerys*, in preparation).

With the latter improvement and in the light of the low cost and the simplicity of reagent preparation, LIA appears to be particularly suitable for routine analysis.

## Acknowledgements

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